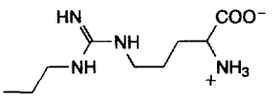
Neuronal nitric oxide synthase inhibitor

Nitric oxide (NO) functions as a hormone and neurotransmitter in a wide range of physiological processes. It is biosynthesized from L-arginine by various NO synthases (NOS), of which three major enzyme isoforms exist: endothelial (eNOS), neuronal (nNOS) and inducible (iNOS). Potent, selective isoform inhibitors may have therapeutic use in the treatment of various disease states including stroke, septic shock, inflammatory arthritis and neurodegeneration.

A recent report by Zhang, H.Q. and coworkers [*J. Med. Chem.* (1997) 40, 3869–3870] describes the discovery that N^{ω}-propyl-L-arginine (**10**) is a highly selective inhibitor of nNOS (K_i = 57 nM) with a 150-fold greater selectivity for nNOS over eNOS and a >3,000-fold selectivity for nNOS over iNOS.



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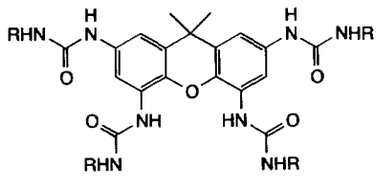
Combinatorial chemistry

DNA-binding compound library

Compounds that inhibit the binding of DNA to transcription factors have been discovered through a combinatorial library that was analysed using a highly efficient deconvolution process [Shipps, G.W. *et al., Proc. Natl. Acad. Sci. U. S. A.* (1997) 94, 11833–11838]. The library was constructed from a central xanthene template derivatized simultaneously with a mixture of amino acids to attach four urea side-chains (**1**). Five sub-



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libraries, each of 2,080 members, were constructed from a mixture of eight amino acids and esters, and the resulting mixtures screened against the transcription factors SpP3A2 and SpZ12-1. Intriguingly, despite the different sequences of the two transcription factors, the libraries appeared to have equivalent activity for both targets.

Following the first round of screening and the identification of one active sublibrary, a process of elimination generated a preferred set of four amino acid residues. Further rounds of deconvolution finally resulted in the identification of two compounds active in the 5–10 μM range.

The selection process used to find these compounds from a library of >10,000 compounds required only 15 separate screening assays. It is thus a highly efficient method for finding active compounds that are presumed to work by intercalation between the DNA basepairs.

Protein tyrosine phosphatase substrates

The phosphorylation of proteins plays a key role in the regulation of a huge range of cellular functions. Consequently, an understanding of the substrate specificity of protein kinases and phosphatases is crucial in the design of drug molecules that can modify these regulatory pathways. In order to define the preferred substrates of the leukocyte antigen receptor (LAR) phosphatase, a combinatorial library of peptides has been prepared [Cheung, Y.W. *et al., J. Am. Chem. Soc.* (1997) 119, 9568–9569]. An especially clever aspect of this study was the use of α-chymotrypsin activity to reveal the preferred substrate sequences.

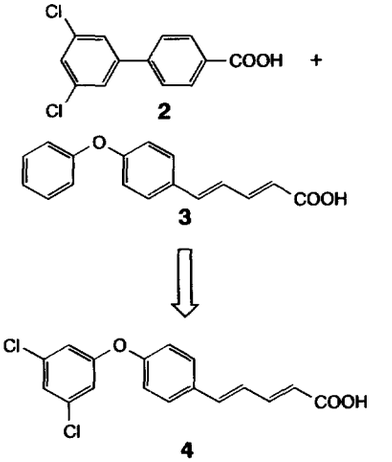
α-Chymotrypsin (CT) cleaves peptides on the C-terminal side of aromatic residues, including tyrosine, but it was reasoned that peptides containing phosphotyrosine would be poor substrates for CT. However, effective substrates for the LAR phosphatase would be converted to good substrates for CT cleavage. Thus, after incubating a resin-bound phosphonopeptide library with LAR phosphatase, the beads were treated with CT, which selected those sequences that had undergone phosphatase-mediated dephosphonation. Beads containing the active sequence were detected by reaction of the newly generated free N-terminus with a chromogenic reagent.

Carrying out this sequence of operations, picking the coloured beads and sequencing their attached peptide led to the recognition of effective substrate sequence motifs for LAR phosphatase.

Human papillomavirus E2 binders

SAR by NMR is a new combinatorial technique by which new pharmacologically active lead molecules can be discovered by screening libraries of compounds in an NMR experiment. Following successes in finding ligands for the FK506 binding protein and for stromelysin, the Abbott group has now used the approach for the DNA-binding domain (DBD) of the human papillomavirus E2 protein [Hajduk, P.J. *et al., J. Med. Chem.* (1997) 40, 3144–3150].

Screening approximately 2,000 small organic molecules against ^{15}N-labelled E2 DBD in a two-dimensional ^{15}N-heteronuclear single-quantum correlation experiment led to the identification of three classes of compound that bound weakly to the protein. Observing the NMR shifts of the protein on binding suggested that two of these compounds bound to the DNA recognition helix,



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whereas the third bound to the β-barrel formed at the interface of the two monomer subunits.

Analogue synthesis allowed optimization of each of these series (to give **2** and **3**). Furthermore, as they bound at distinct sites on the E2 protein, it was possible to combine both motifs into a single molecule (**4**). In this way, the compounds first discovered that bound with mM affinity were optimized to a 10 μM ligand suitable as a lead for further studies towards an antiviral agent for human papillomavirus.

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Emerging molecular targets

A key to Alzheimer's disease?

Marijuana

Researchers have known for a long time that the use of marijuana is associated with memory impairment. One striking feature of marijuana is that short-term memory, required for immediately recalling lists of objects, is badly hampered, whereas long-term memory, used for recalling past events and historic facts, is not affected. This situation is reminiscent of senile dementia, in particular Alzheimer's disease, except that the memory retention effects of marijuana use are completely reversible. It has also been known for some time that 9-Δtetrahydrocannabinol, the active ingredient in marijuana, blocks long-term potentiation (LTP) in the hippocampus CA1 region of the brain, an area implicated in short-term memory processing.

2-Arachidonylglycerol

Recently, Dr Daniele Piomelli and coworkers at the Neurosciences Institute (San Diego, CA, USA) have identified an endogenous brain cannabinoid, 2-arachidonylglycerol (2-AG), which is produced in electrically stimulated brain slices in a tetrodotoxin-sensitive, calcium-dependent fashion, activates cannabinoid CB1 receptors, and prevents LTP at CA3/CA1 synapses [*Nature* (1997) 388, 773–778]. The original identification of 2-AG as an endogenous cannabinoid agonist was made in 1995 in the canine gut by Raphael Mechoulam and his colleagues from the Hebrew University (Jerusalem, Israel) The new study, however, embraces the required criteria for establishing the role of 2-AG as an endogenous brain neuromodulator.

Biochemical synthesis

The identification of 2-AG as an endogenous brain cannabinoid follows the identification, several years ago, of the first endogenous brain cannabinoid ligand, anandamide. However, unlike anandamide, which is formed from phospholipids via the action of phospholipase D, 2-AG is formed from phospholipids via the more ubiquitous phospholipase C (PLC) pathway, employed by numerous G-protein-coupled neurotransmitter receptors. Several hormones and peptides, such as thrombin and endothelin, also utilize the PLC pathway. This pathway crosslinks with the protein kinase C (PKC) signalling pathway, as its primary product, diacylglycerol (DAG), is both an activator of PKC and a precursor for 2-AG. Another PLC product, inositol 1,4,5-triphosphate, mediates Ca^{2+} release from intracellular stores, thereby affecting 2-AG production from DAG by the calcium-dependent DAG lipase.

Most notably, 2-AG reaches much higher brain levels, ~170-fold, than anandamide (~4 nmol g^{-1} tissue compared with 23 pmol g^{-1} tissue). The identification of 2-AG as the major endogenous brain cannabinoid agonist may thus herald a new route for designing memory-enhancing drugs. Indeed, selective CB1 antagonists, such as Sanofi Research SR141716A, were recently shown to possess distinct memory-enhancing properties [Terranova, J.P. *et al.*, *Psychopharmacology* (1996) 126, 165–172]. It remains to be seen whether 2-AG is elevated in memory-impaired individuals. However, the rapid turnover of 2-AG may prohibit human post-mortem studies (Piomelli's group used rapid freezing to measure brain 2-AG levels). Studies in animal models of Alzheimer's disease will ultimately be required to assess whether 2-AG has a plausible role in memory processing and memory impairment.

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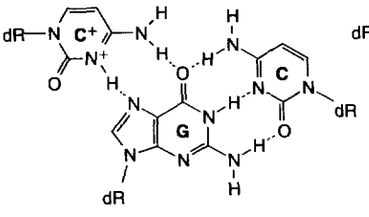
Erratum

In the January Monitor section, the Profile article entitled *Antigene oligonucleotides* by Wentland, M.P. [*Drug Discovery Today* (1998) 1, 45] showed the structures of two nucleotide triplexes. These were incorrect and should have appeared as shown. We apologise to the author and the readers of the journal for this error.



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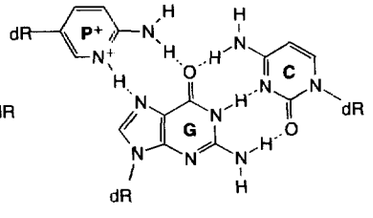

18 P^+ - - G - C

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